

ORIGINAL ARTICLE

Gut bacteria comparison between wild and captive neotropical otters

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Abstract

The neotropical otter (Lontra longicaudis) is considered a flagship species for the conservation of the ecosystems in which it resides and is currently in a vulnerable state. As a conservation strategy for this species, rehabilitation, breeding, and reintroduction programs of captive individuals have been proposed. However, it is likely that the environment and feeding conditions in captivity result in gut microbial communities that differ from those in wild animals. Gut microbial communities have an important role in the physiological performance of an animal. To determine differences between gut microbial communities of otters in wild and captive living conditions, the structure and diversity of their gut bacterial communities were determined using 16S rDNA molecular markers. Total DNA was isolated from fecal samples of wild animals from the La Vieja River basin and from captive animals in the Cali Zoo. As expected, the gut bacterial communities of captive animals converged to a more similar structure, and their bacterial diversity was significantly lower than that found in wild animals.

Keywords: Gut bacterial community; *Lontra longicaudis*; PCR-DGGE molecular profile; wild and captive otters.

Introduction

The neotropical otter, *Lontra longicaudis*, is considered a flagship species for the conservation of the ecosystems in which it resides; its presence indicates high energy availability and biodiversity [1, 2]. This species is distributed throughout Central and South America, inhabiting coastal environments, mangrove zones, arid zones with thorny forests and shrubs, marshy areas, tropical forests, and sub-Andean forests [3]. The neotropical otter currently



has the conservation status of vulnerable in Mexico, Venezuela, Colombia, Belize, and Ecuador. This is a result of the environmental changes generated by anthropogenic disturbances and the increase in the incidence of bacterial infections within its populations [4, 5]. It is estimated that the number of individuals of the species will decline by 25 % in the next 27 years [6].

Breeding and rearing otters in captivity is one strategy to mitigate the decline that is currently observed in populations of several otter species around the world including *L. longicaudis* [7]. Therefore, it is important to seek the continuous improvement of management practices in captive breeding programs. Diet is one aspect of management for which information is still needed to improve living conditions in captivity. Diet is important not only because it supports an animal 's nutritional demands for growth and reproduction, but also it is one of the determining factors in the composition of gut microbiota [8]. In turn, gut microbiota are intimately linked to the physiology of the animal and are closely related to brain function, immune system response, digestion and absorption of nutrients, control of the body 's inflammatory pathways, and neutralization of food toxins [9-16]. Consequently, changes in the intestinal microbiota may have potential effects on the animal's fitness [16, 17].

Previous studies on different animal species indicate that captive breeding programs involve changes in the animal's diet that may affect the internal microbial ecology of the gut [18-22]. Comparative studies that focus on a particular species showed a decrease in the gut microbial diversity of captive animals compared to animals in the wild [23, 24]. However, a study in six orders of mammalian taxa concluded that the effect of captivity is host-specific [22], revealing no microbial diversity differences between animals in wild and captive conditions for bovids, giraffes, anteaters, and aardvarks; but, reporting reductions in captive canids, primates, and equids. On the other hand, a study on woodrats (*Neotoma sp.*) [25] indicated that the hosts most susceptible to experience a decrease in the diversity of their gut microbiota are those with a specialized diet [26-28].

Considering the influence that gut microbiota may have on an animal's fitness, we investigated the effect that captivity has on the otter's gut microbiota. This is a relevant topic because rearing conditions in captivity could affect the success of reintroduction of the animal into the wild [29, 30]. Currently, there are no characterizations of the gut microbiota in the neotropical otter, making necessary a baseline of knowledge to support future evaluations of the captivity effects in the animal's gut microbiota and its relation to physiological performance. Therefore, the objective of this work was to

contrast the structure and diversity of the gut bacterial communities in wild and captive neotropical otters. We found that the gut bacterial community of captive animals converged to a more similar structure and diversity. The environment in which the animals live and the low variation in the supplied food [31], may be affecting the gut microbiota of captive otters.

Materials and Methods

The diversity and structure of the gut bacterial microbiota in wild and captive otters were evaluated through the genetic markers, namely the 16S rDNA region of fecal bacteria. Total DNA was isolated from fecal samples deposited on the ground by wild animals that inhabited the La Vieja River basin (in central western Colombia) and by captive animals in the Cali Zoo (Valle del Cauca, Colombia), an institution that has been working in the field of reproduction of captive otters for two decades. Fecal sampling is a noninvasive method that serves as a proxy to study the microbial composition of the large intestine [32].

Sample collection

A total of eight fresh fecal samples from wild animals, characterized by large quantities of spines and/or scales, fishy odor and greenish secretion with liquid consistency [33, 34], were collected in a 10 km boat sailing along the La Vieja River basin (Fig. 1). This green liquid evaporates completely within an hour and a half of being evacuated by the animal [Personal observation]. In this study, we only collected samples in which this green liquid was still present. Upon finding a fresh sample, a portion of approximately 15 g was extracted from the center of the fecal mound with a sterile flat stick, excluding matter in contact with the environment. Subsequently, the sample was identified and stored in airtight bags at 4 °C. At the end of the 10 km transect, the samples were transported to the laboratory and stored at -27 °C until analysis. It is not known how many different wild animals the collected samples came from. However, considering the dispersal radius of the neotropical otter in the La Vieja River basin and the location of the sampled fecal matter [35], it is estimated that these samples could come from three different animals.

In the Cali Zoo, fecal samples were collected from the animal enclosures following the procedure described above. Each fecal sample was left one hour after being evacuated under the weather of the enclosure area in order to simulate the collection conditions of samples from wild animals. Given the captivity conditions, 12 samples were collected from three different otters on different days. These otters were living together in the same enclosure.



Figure 1. Location of sampling sites on the map of Colombia, South America.

In both, La Vieja River basin and the Cali Zoo, the samples were collected between 9 and 12 pm because this was the time window in which it was possible to observe fresh fecal matter. The number of samples per sampling site, sampling date, and sampling location coordinates are reported in **Table 1**. Location of sampling sites are shown on the map of Colombia, South America (Fig. 1).

Nucleic acid analysis

Total DNA was extracted in triplicate from each fecal sample. The similarities among the PCR-DGGE banding patterns obtained from these three

Sampling

site

Sample

ID

r basin (VR) and the Cali Data not available.					
Animal source	Sampling date				
n/a	25-03-16				
n/a	25-03-16				

Table 1. Fecal samples collected in the La Vieja River basin (VR) and the Ca	li
Zoo (CZ). (FS) = Fecal sample, $O = Otter$, $(n/a) = Data not available$.	

Sampling point

(Coordinates)

La Vieja River basin	VR_FS 1	N 4° 46' 0.466'' W 75° 50' 44.545''	n/a	25-03-16
	VR_FS 2	N 4° 46' 14.498'' W 75° 50' 46.672''	n/a	25-03-16
	VR_FS 3	N 4° 46' 31.458'' W 75° 50' 53.962''	n/a	25-03-16
	VR_FS 4	N 4° 46' 41.977" W 75° 51' 18.842"	n/a	25-03-16
Feces from wild animals	VR_FS 5	N 4° 46' 41.912'' W 75° 51' 18.874''	n/a	25-03-16
	VR_FS 6	N 4° 46' 31.901" W 75° 52' 20.535"	n/a	25-03-16
	VR_FS 7	N 4° 46' 31.836'' W 75° 52' 20.6''	n/a	26-03-16
	VR_FS 8	N 4° 45' 58.007" W 75° 52' 43.572"	n/a	26-03-16
Cali Zoo	CZ_FS 1	All the samples were collected at N 3° 26' 54.041'' W 76° 33' 24.932''	O_1	02-06-16
	CZ_FS 2		O_1	07-06-16
	CZ_FS 3		O_1	09-06-16
	CZ_FS 4		O_1	13-06-16
Feces from captive animals	CZ_FS 5		O_1	15-06-16
	CZ_FS 6		O_2	01-06-16
	CZ_FS 7		O_2	03-06-16
	CZ_FS 8		O_2	05-06-16
	CZ_FS 9		O_2	07-06-16
	CZ_FS 10		O_3	05-06-16
	CZ_FS 11		O_3	08-06-16
	CZ_FS 12		O_3	15-06-16

subsamples were used to assess the spatial heterogeneity of the bacterial community composition in a particular fecal sample. Afterwards, the genetic profiles obtained from the different samples were compared.

DNA extraction and molecular analysis

The total DNA in 0.5 g of each fecal subsample was isolated as described in Santamaría *et al.* [36]. Later, a first PCR was performed with the 16S rDNA universal primers 8F 5'-AGA GTT TGA TCC TGG CTC AG-3'and 1541R 5'-AAG GAG GTG ATC CAG CCG CA-3' to assess the quality of the isolated total DNA. Next, the oligonucleotides 968F-GC 5'-CGC CCG CCG CGC GCG GCG GGC GGC GGG GCG GGG GCA CGG GGGG AAC GCG AAG AAC CTT AC-3 'and 1401R 5'- CGG TGT GTA CAA GAC CCG GGA ACG-3' [36] were used to amplify a 433 bp long region of the 16S rDNA using the isolated total DNA as a template. These 433 bp amplification products were the fragments analyzed by Denaturing Gradient Gel Electrophoresis-DGGE. Two PCRs were carried out, with each isolated total DNA as technical replicates.

Amplification reactions were carried out as in Santamaría *et al.* [36]. PCR thermocycling program for the 8F and 1541R primers were the same as in Lofler *et al.* [37]. PCR cycling temperatures for the 433 bp fragment amplification with the 968F and 1401R primers were as follows: 94 °C for 3 min (1 cycle); 94 °C for 50 s, 48 °C for 20 s, and 72 °C for 45 s (30 cycles); followed by a final step at 72 °C for 5 min. The DGGE analysis of the 433 pb PCR products was performed according to Santamaría *et al.* [38], using a different denaturing gradient (37 % to 65 %) and an electrophoresis run time of 17 h at 35 V and 60 °C.

Diversity and structure of bacterial communities

It is impossible to determine whether more than one fecal sample came from the same animal in the wild; therefore, each of the fecal samples collected in this study, including samples of animals in captivity, was treated as an independent sample.

To compare the gut bacterial community between wild and captive otters, a UPGMA dendogram was created from a similarity matrix, which was calculated with the jaccard Index by comparing the 16S rDNA DGGE samples profiles. Comparisons among triplicates from each fecal sample were carried out in the same way. Larger differences between wild and captive otters than differences within each group, were evaluated with an analysis of similarity test (ANOSIM) using the animals' condition (captive and wild) as a grouping factor.

The abundance of each the fragments produced by PCR-DGGE does not mirror the abundance of different microbial species in a sample, mainly because the same set of primers amplify different DNA templates with variable efficiencies. Thence, in this study, richness (S, equation 1) was used to estimate the gut bacterial community diversity in the collected samples to avoid a diversity evaluation based on a biased PCR abundance estimation [39, 40].

$$S = \Sigma bands present in a sample \tag{1}$$

The S value was calculated from a binary table that shows the presence or absence of a particular band observed in the DGGE banding profile. This table was built using the Gel Compare II 5.0, Applied Maths NV software (Sint-Martens-Latem, Belgium).

The difference between the S values of gut bacterial communities from wild and captive animals was evaluated with a one-way ANOVA test. This analysis used the average richness value calculated from the three subsamples of each fecal sample. Data normality (W = 0.94, p > 0.05) and homogeneity of variances were evaluated with the Shapiro-Wilk and Levene tests.

As stated before, each sample was treated as an independent sample. However, when assuming that all samples are independent there is a likelihood that the results are over inflated because the models do not control for repeated sampling. Hence, the scenario that the fecal samples from wild otters come from three different animals was also considered, and a second ANOVA analysis was performed to compare the average bacterial diversity in the samples from each of the three animals in captivity with that of samples from the three possible wild individuals. So, the eight fecal samples collected from wild otters were randomly organized and averaged to reflect three different animals. Four different set combinations of the fecal samples were evaluated.

The XLSTAT software (MS Excel, Addinsoft, NY, USA) was used for the clustering and ANOVA analyses. The R package Vegan was employed for the ANOSIM. For all statistical tests, p < 0.05 was the applied significance level.

Results and Discussion

The 433 pb 16S rDNA amplification products were obtained from all evaluated total DNA isolates except from subsamples 2 and 3 from fecal sample (FS) 8 collected at the La Vieja River (Table 1).

Bacterial composition similarities among fecal subsamples

The DGGE profiles obtained from the three subsamples of each captive FSs were identical in 83 % of the cases (Fig. 2). The exceptions were subsample 1 from FS 2, with a similarity of 86 % to the other two subsamples, and



Figure 2. A) DGGE banding profiles of the fecal samples coming from captive otters in the Cali Zoo (CZ) and B) their respective clustering ordination data. (O) = Otter, (FS) = Fecal sample, (1, 2, 3) = Indicate the three bands in the DGGE image associated with each fecal sample. Each band shows the genetic profile of each subsample.

subsample 2 from FS 8, with a similarity of 88 %. In both cases, the other two subsamples were 100 % similar to each other. This result suggests that the bacterial species composition is relatively homogeneous throughout the FSs of captive otters. Sample spatial heterogeneity in relation to species composition was evident in FSs collected from wild animals. In this case, the DGGE profiles among subsamples of the same FS were more dissimilar. Data obtained from wild animals revealed that 75 % of the FSs had similarities between 85 % and 94 % among their subsamples. Only 25 % of the FSs coming from wild otters showed a 100 % DGGE profile similarity among their subsamples (**Fig. 3**). This detected heterogeneity among subsamples may indicate spatial variability of the bacterial community in the fecal sample and is in agreement with information reported for healthy humans where the microbiota was

However, this spatial heterogeneity may also be an artifact of the sample collection. Considering that each FS from La Vieja River was exposed to the environment for a unique amount of time (between minutes and one hour and half) before being collected and stored at 4 °C, bacterial community

not equally distributed within feces [41].



Figure 3. A) DGGE banding profiles of the fecal samples from wild otters in the La Vieja River basin (VR) and B) their respective clustering ordination data. (FS) = Fecal sample, (1, 2, 3) = Indicate the three bands in the DGGE image associated with each fecal sample. Each band shows the genetic profile of each subsample.

composition is likely to differ among these FSs. This could also explain why this variability was not observed in the subsamples of the captive animals where all fecal samples were collected after one hour and under the same environmental conditions after being evacuated within the animal enclosures.

Bacterial composition heterogeneity among fecal samples

The cluster analysis also shows ample variation, between 26 % and 64 %, in the bacterial community similarity values among the FSs from wild animals (Fig. 3B). Assuming that the FSs come from different wild otters, the differences may be driven by factors such as age, body mass index, and health status of the animals from which the samples came. Furthermore, environmental differences such as geographic location and diet play also a role, as has been previously established for humans and other animal species [42-49]. These samples could also come from three different animals, that may belong to different social groups. The possibility that the observed heterogeneity among samples may be the result of shifts in the bacterial community of the fecal sample after being evacuated by the animal cannot be ruled out (see preceding section)

Bacterial communities among FSs from captive otters exhibited more similarities that those from wild animals. The range of bacterial community similarities spanned 43 % and 87 % (Fig. 2B), which could be an effect of these three animals living within the same enclosure as one social group, and the fact that all samples were collected exactly one hour after being evacuated by the animal.

Data from captive animals also revealed bacterial composition variation higher than the expected among samples from the same animal, as evidenced by FSs 1, 2, 3, 4, and 5 from the otter identified as O_1 in the Cali Zoo, with bacterial community similarities ranging from 41 % to 86 %. The same trend was observed for samples collected from the animals identified as O_2 and O_3 (Fig. 2B). It is unlikely that differences between fecal samples coming from the same animal are the result of spatial heterogeneity in the fecal sample because, as previously shown, bacterial species composition is relatively homogenous in most of the analyzed FSs from captive otters. These differences among FSs from the same animal are the likely result of a normal temporal variation in the bacterial composition of the large intestine.

Although it has been established that the microbiome of a mammal remains relatively stable over time [50, 51], there are normal levels of temporal variability in the microbial composition [52]. It has been reported that the

gut microbiome studied through fecal samples showed variation between medium (2-4) and long-term sampling periods (>1 month) [53]. However, a more recent study on spatiotemporal dynamics using replicate sampling, showed daily temporal changes in the collected FSs [54]. These fast changes may reflect a microbial succession characterized by a sequential dominance of microorganisms in which the consumption of readily fermentable substrates during passage through the large intestine drives microbial metabolism to shift from a saccharolytic fermentation metabolism to a proteolytic one [55]. This process could also explain the variability observed among samples from wild otters in cases where some feces came from the same animal.

Bacterial community structure in samples from wild vs captive otters

When all collected samples were analyzed together, two gut bacteria groups were distinguished with the UPGMA approach (**Fig. 4**). These two groups shared 20 % of their bacterial composition and corresponded to gut bacteria from wild and captive otters. The ANOSIM analysis confirmed the grouping



Figure 4. Clustering ordination data of the 16S rDNA DGGE banding profiles from fecal subsamples collected from captive otters in the Cali Zoo (CZ) and wild animals in the La Vieja River basin (VR). (O) = Otter, (FS) = Fecal sample, (1, 2, 3) = Indicate the subsamples of each fecal sample.

of the samples coming from animals under the same living conditions (R=0.6752, p < 0.001), suggesting important differences between bacterial communities in the fecal matter of captive and wild animals.

Fig. 4 also shows the formation of subgroups within both the captive and the wild groups. The significance of these subgroups was evaluated by a different ANOSIM test for each wild and captive group using "animal" and "fecal sample" as grouping factors for captive otters and "fecal sample" as the grouping factor in samples from wild animals. The results confirmed differences only among subgroups in the wild (R = 0.1835, p < 0.05). This finding confirms the observations pointing at higher bacterial composition similarity among fecal samples from captive otters compared to that of samples from wild otters. Taking into account a possible sampling effect, subgroups formed by samples from wild animals may indeed correspond to different animals, who may or may not belong to the same social group, and who are living in a more diverse environment than the one captive otters experience. The homogeneity among the samples of captive animals probably reflects the captivity condition. In the zoo, animals live in small areas interacting with a less dynamic environment, contrary to what wild animals experience.

Effect of captivity on gut bacterial diversity

Gut bacterial diversity in captive otters was reduced compared to that in wild animals. Richness data (S) complied with the assumptions of normality (W = 0.94, p > 0.05) and homogeneity of variances (F = 3.8, p > 0.5); therefore, these data were analyzed via one-way ANOVA. The genetic polymorphism detected by PCR-DGGE in the gut bacterial community of wild animals showed a higher richness index (15 ± 3.5) (F = 33.6, p < 0.0001) than the one estimated for those in captivity (8 ± 1.7) (Fig. 5). A second ANOVA analysis was performed to compare the fecal samples of the three captive animals with different set combinations of fecal samples representing three wild animals (data not shown). This analysis also showed significant differences between wild and captured animals (F = 62.48, P < 0.0001). This result coincides with reports of a lower diversity in the gut microbial community of animals in captivity [56-58], especially if the animals have a diet with limited food diversity [54, 59]. The diet of the neotropical otter living in the La Vieja River basin is based mainly on fish (76.7%), mostly of the Loricariidae family, followed by insects (12.67 %) and reptiles (0.7 %) [60]. In contrast, in the zoo, the diet is based mainly on a single species, Oreochromis niloticus from the Mojarra family, which is supplied alive or frozen. Occasionally, animals are also offered coconut.



Figure 5. Diversity of bacterial gut communities as evaluated by richness (S). The diversity value presented in the graph is the average calculated from the eight fecal samples collected from wild otters in the La Vieja River basin and the twelve fecal samples collected from captive otters in the Cali Zoo. (*) Indicates a significant difference in richness between the communities of the wild and captive animals (p < 0.0001).

Despite the fact that in the present study the greater diversity observed in wild otters coincides with that reported in the literature for other species, these results from the animals at La Vieja River and the Cali Zoo should be viewed with caution because the fecal samples were not collected immediately after being evacuated by the animal. The effect thereof on the diversity and structure of the bacterial community cannot be determined. Although the fecal samples spent a relatively short time in the open before being sampled, this may cause a shift in the composition of the microbial populations and also the decomposition of the DNA through enzymatic degradation, oxidation, and hydrolysis. [61, 62]. It is also necessary to keep in mind that the DGGE technique provides only a rough estimate of the richness and structure of microbial populations.

Given the effect of captivity reflected on our study's results, it is worth continuing research on the gut microbiota of the neotropical otter to better understand the implications captivity diet may have on individual fitness and to foresee the success of these animals within wild reintroduction programs. Subsequent studies must take in consideration the analysis of fresh samples (i.e. collected immediately after being evacuated) by using metagenomic sequencing that allow gut bacteria taxa identification. Future studies with metagenomics, identification, and monitoring of wild animals and more sampled animals from different social groups of captive animals, should provide detailed information about gut bacteria community composition, their temporal variation and their differences among individuals. New research must also consider the evaluation of the possible role of the gut microbial diversity and community structure to set clear if these have an important function in the otter's physiology.

Conclusion

The similarity values obtained when comparing both samples and subsamples and the richness analysis showed that the species composition of bacterial communities in captive animals tended to be more homogeneous and less diverse than those in wild animals. Furthermore, bacterial community structure differed between fecal samples from animals living in the wind and in captivity.

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Conflict of interest

The authors declare that this work does not present any conflict of interest.

References

[1] Gallo-Reynoso JP, Ramos-Rosas NN, Rangel-Aguilar Ó. Depredación de aves acuáticas por la nutria neotropical (*Lontra longicaudis* annectens), en el río Yaqui, Sonora, México. Revista Mexicana de Biodiversidad, 79: 275–279, 2008.

doi: 10.22201/ib.20078706e.2008.001.502

[2] Fundación Omacha, Ministerio de Ambiente y Desarrollo Sostenible. Plan de manejo para la conservación de las nutrias (Lontra longicaudis y Pteronura brasiliensis) en Colombia, (2016).

https://www.minambiente.gov.co/images/ BosquesBiodiversidadyServiciosEcosistemicos/pdf/ Programas-para-la-gestion-de-fauna-y-flora/plan-manejoconservacion-nutrias-colombia-final.pdf

[3] Rodrigues L de A, Leuchtenberger C, Kasper CB, Junior OC, Fonseca da Silva VC. Avaliação do risco de extinção da lontra neotropical *Lontra longicandis* (Olfers, 1818) no Brasil. *Biodiversidade Brasileira*, 3: 216-227, 2013.

http://www.icmbio.gov.br/revistaeletronica/index.php/ BioBR/article/view/389

[4] Rheingantz ML, Trinca CS. Lontra longicaudis. The IUCN Red List of Threatened Species 2015.3

doi: 10.2305/IUCN.UK.2015-2.RLTS.T12304A21937379.en

[5] Corporación Autónoma Regional del Valle del Cauca. Nutria de río: planes de manejo para 18 vertebrados amenazados del departamento del Valle del Cauca. 2007

https://www.researchgate.net/profile/Isabel_Avila2/ publication/293606223_Planes_De_Manejo_Para_18_ Vertebrados_Amenazados_Del_Departamento_Del_Valle_ Del_Cauca/links/56f5b8b108ae7c1fda2eeb56/Planes-De-Manejo-Para-18-Vertebrados-Amenazados-Del-Departamento-Del-Valle-Del-Cauca.pdf

[6] Pacifici M, Santini L, Di Marco M, Baisero D, Francucci L, Grottolo Marasini G, Rondinini C. Generation length for mammals. *Nature Conservation*, 5: 89-94, 2013.

doi: 10.3897/natureconservation.5.5734

[7] Reed-Smith J, Larson S. Otters in Captivity. In: Butterworth A. (Eds), Marine Mammal Welfare. Animal Welfare, 52: 573-584, 2017.

doi: 10.1007/978-3-319-46994-2_31

[8] Groussin M, Mazel F, Sanders JG, Smillie CS, Lavergne S, Thuiller W, Alm E. Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nature Communications*, 8: 14319, 2017.

doi: 10.1038/ncomms14319

[9] Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B. Normal gut microbiota modulates brain development and behavior. *Proceedings of the Natural Academy of Sciences of the United States of America*, 108: 3047-3052, 2001.

doi: 10.1073/pnas.1010529108

[10] Hooper LV. Do symbiotic bacteria subvert host immunity? *Nature Reviews Microbiology*, 7: 367-375, 2009.

doi: 10.1038/nrmicro2114

[11] Amato KR, Leigh SR, Kent A, Mackie RI, Yeoman CJ, Stumpf RM, Garber PA. The role of gut microbes in satisfying the nutritional demands of adults and juveniles wild, black howler monkeys (*Alouatta pigra*). *American Journal of Physical Anthropology*, 155: 652-664, 2014.

doi: 10.1002/ajpa.22621

[12] Hooper LV, Midtvedt TM, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annual Review of Nutrition*, 22: 283-307, 2002.

doi: 10.1146/annurev.nutr.22.011602.092259

[13] Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*, 489: 242-249, 2012.

doi: 10.1038/nature11552. 10

[14] Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, 453: 620-625, 2008.

doi: 10.1038/nature07008

[15] Claus S. Guillou H. Ellero-Simatos S. The gut microbiota: a major player in the toxicity of environmental pollutants? *Npj Biofilms And Microbiomes*, 2: 16003, 2016.

doi: 10.1038/npjbiofilms.2016.3

[16] Suzuki TA. Links between natural variation in the microbiome and host fitness in wild mammals. *Integrative and Comparative Biology*, 57: 756-769, 2017.

doi: 10.1093/icb/icx104

[17] Amato KR. Co-evolution in context: The importance of studying gut microbiomes in wild animals. *Microbiome Science and Medicine*, 1: 10-29, 2013.

doi: 10.2478/micsm-2013-0002

[18] Schwab C, Gänzle M. Comparative analysys of fecal microbiota and intestinal microbiota metabolic activity in captive polar bears. *Canadian Journal of Microbiology*, 57: 177-185, 2011.

doi: 10.1139/W10-113

[19] Schwab C, Cristescu B, Northrup JM, Stenhouse GB, Gänzle M. Diet and environment shape fecal bacterial microbiota composition and enteric pathogen load of grizzly bears. *PLoS ONE*, 6: e27905, 2011.

doi: 10.1371/journal.pone.0027905

[20] Robles-Alonso V, Guarner F. Progress in the knowledge of the intestinal human microbiota. *Nutrición Hospitalaria*, 28: 553-7, 2013.

doi: 10.3305/nh.2013.28.3.6601

[21] Delport TC, Power ML, Harcourt RG, Webster KN, Tetu SG. Colony location and captivity influence the gut microbial community composition of Australian sea lion (*Neophoca cinerea*). *Applied and Environmental Microbiology*, 82: 3440-3449, 2016.

doi: 10.1128/AEM.00192-16

[22] McKenzie VJ, Song SJ, Delsuc F, Prest TL, Oliverio AM, Korpita TM, Alexiev A, Amato KR, Metcalf JL, Kowalewski M, Avenant NL, Link A, Di Fiore A, Seguin-Orlando A, Feh C, Orlando L, Mendelson JR, Sanders J, Knight R. The effects of captivity on the mammalian gut microbiome. *Integrative and Comparative Biology*, 57: 690-704, 2017.

doi: 10.1093/icb/icx090

[23] Lavery TJ, Roudnew B, Deymour J, Mitchell JG, Jeffries T. High nutrient transport and cycling potential revealed in the microbial metagenome of Australian sea lion (*Neophoca Cinerea*) Faeces. *PLoS ONE*, 7: e36478, 2012.

doi: 10.1371/journal.pone.0036478

[24] Zhu L, Wu Q, Dai J, Zhang S, Wei F. Evidence of cellulose metabolism by the giant panda gut microbiome. Proceedings of the National Academy of Sciences of the United States of America, 108: 17714-19, 2011.

doi: 10.1073/pnas.1017956108

[25] Kohl KD, Skopec MM, Dearing MD. Captivity results in disparate loss of gut microbial diversity in closely related hosts. *Conservation Physiology*, 2: cou009, 2014.

doi: 10.1093/conphys/cou009

[26] Ley UE, Hamady M, Luzupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI. Evolution of mammals and their gut microbes. *Science*, 320: 1647-51, 2008.

doi: 10.1126/science.1155725

[27] Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, Henrissat B, Knight R, Gordon JI. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, 332: 970-74, 2011.

doi: 10.1126/science.1198719

[28] Delsuc F, Metcalf JL, Wegener PL, Song SJ, González A, Knight R. Convergence of gut microbiomes in myrmecophagous mammals. *Molecular ecology*, 23: 1301-17, 2014.

doi: 10.1111/mec.12501

[29] Bahrndorff S, Alemu T, Alemneh T, Nielsen JL. The Microbiome of animals: implications for conservation biology. *International Journal of Genomics*, Article ID 5304028, 2016.

doi: 10.1155/2016/5304028

[30] Trevelline BK, Fontaine SS, Hartup BK, Kohl KD. Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings Of The Royal Society B. Biological sciences*, 286:20182448, 2019.

doi: 10.1098/rspb.2018.2448

[31] Heiman ML, Greenway FL. A healthy gastrointestinal microbiome is dependent on dietary diversity. *Molecular Metabolism*, 5: 317-320, 2016.

doi: 10.1016/j.molmet.2016.02.005

[32] Seekatz AM, Schnizlein MK, Koenigsknecht MJ, Baker JR, Hasler WL, Bleske BE, YoungVB, Sun D. Spatial and temporal analysis of the stomach and small-intestinal microbiota in fasted healthy humans. *mSphere*, 4: e00126-19, 2019.

doi: 10.1128/mSphere.00126-19

[33] Álvarez-León R. Importancia de los peces en la nutrición de la nutria gigante de río (*Pteronura brasiliensis*) (Carnívora, *Mustelidae*) en Colombia. Revista Luna Azul, 28: 8-14, 2009.

http://www.scielo.org.co/pdf/luaz/n28/n28a01.pdf

[34] Chemes SB, Giraudo AR, Gil G. Dieta de Lontra Longicaudis (Carnivora, Mustelidae) En El Parque Nacional El Rey (Salta, Argentina) y Su Comparación Con Otras Poblaciones de La Cuenca Del Paraná. Mastozoología neotropical, 17: 19-29, 2010.

http://www.scielo.org.ar/scielo.php?script=sci_ arttext&pid=S0327-93832010000100003&lng=es&nrm=iso&tl ng=es

[35] Portocarrero A, Morales D, Diaz D. Nutrias de colombia. Fundación Omacha- Fundación Horizonte Verde. Proyecto Pijiwi. orinoko. 2009.

https://www.academia.edu/28752848/Nutrias_de_Colombia. pdf

[36] Santamaría J, López L, Soto CY. Detection and diversity evaluation of tetracycline resistance genes in grassland-based production systems in Colombia, South América. *Frontiers in Antimicrobials, Resistance and Chemotherapy*, 2: article 252, 2011.

doi: 10.3389/fmicb.2011.00252

[37] Loffler FE, Sung QJ, Li J, Tiedje JM. 16S rRNA gene based detection of tetrachloroethene dechlorinating Delsufuromonas and Dehalococcoides species. *Applied and Environmental Microbiology*, 66: 1369-1374, 2000.

doi: 10.1128/AEM.69.2.996-1003.2003

[38] Santamaría J, Parrado CA, López L. Soil Microbial Community Structure and Diversity in Cut Flower Cultures Under Conventional and Ecological Management. *Revista Brasileira de Ciencia do Solo*, 42: e0170016, 2018.

doi: 10.1590/18069657rbcs20170016

[39] Weller DM, Raaaijmakers JM, Gardener BBM, Thomashow LS. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, 40: 3019-48, 2002.

doi: 10.1146/annurev.phyto.40.030402.110010

[40] Cedeño R. Caracterización de comunidades bacterianas en sistemas de engorde de camarón mediante electroforesis en geles de gradiente denaturante (DGGE). *Cenaim Informa*, 133: 593-4, 2005.

http://www.revistaaquatic.com/documentos/docs/bquinc133.pdf

[41] Swidsinski A, Loening-Baucke V, Verstraelen H, Osowska S, Doerffel Y. Biostructure of fecal microbiota in healthy subjects and patients with chronic idiopathic diarrhea. *Gastroenterology*, 135: 568-579, 2008.

doi: 10.1053/j.gastro.2008.04.017

[42] Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, Marchesi JR, Falush D, Dinan T, Fitzgerald G, Stanton C, van Sinderen D, O'Connor M, Harnedy N, O'Connor K, Henry C, O'Mahony D, Fitzgerald AP, Shanahan F, Twomey C, Hill C, Ross RP, O'Toole PW. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences of the United States of America*, 108 Suppl 1:4586-4591, 2011.

doi: 10.1073/pnas.1000097107

[43] Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto J, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen H, Juncker A, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal E, Brunak S, Clément K,, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, De Vos W, Zucker J, Raes J, Hansen T, Bork P, Wang J, Ehrlich S, Pedersen O, Guedon E, Delorme C, Layec S, Khaci G, Van De Guchte M, Vandemeulebrouck G, Jamet A, Dervyn R, Sanchez N, Maguin E, Haimet F, Winogradski Y, Cultrone A, Leclerc M, Juste C, Blottière H, Pelletier E, Lepaslier D, Artiguenave F, Bruls T, Weissenbach J, Turner K, Parkhill J, Antolin M, Manichanh C, Casellas F, Boruel N, Varela E, Torrejon A, Guarner F, Denariaz G, Derrien M, Van Hylckama Vlieg J, Veiga P, Oozeer R, Knol J, Rescigno M, Brechot C, M'Rini C, Mérieux A, Yamada T. Richness of human gut microbiome correlates with metabolic markers. Nature, 500: 541-546, 2013.

doi: 10.1038/nature12506

[44] Cotillard A, Kennedy S, Kong L, Prifti E, Pons N, Le Chatelier E, Almeida M, Quinquis B, Levenez F, Galleron N, Gougis S, Rizkalla S, Batto J, Renault P, Doré J, Zucker J, Clément K, Ehrlich S, Blottière H, Leclerc M, Juste C, De Wouters T, Lepage P, Fouqueray C, Basdevant A, Henegar C, Godard C, Fondacci M, Rohia A, Hajduch F, Weissenbach J, Pelletier E, Le Paslier D, Gauchi J, Gibrat J, Loux V, Carré W, Maguin E, Van De Guchte M, Jamet A, Boumezbeur F, Layec. Dietary intervention impact on gut microbial gene richness. *Nature*, 500: 585-588, 2013.

doi: 10.1038/nature12480

[45] Wu G, Chen J, Hoffmann C, Bittinger K, Chen Y, Keilbaugh S, Bewtra M, Knights D, Walters W, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman F, Lewis J. Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 334: 105-108, 2011.

doi: 10.1126/science.1208344

[46] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505: 559-563, 2014.

doi: 10.1038/nature12820

[47] Gilbert JA, Quinn RA, Debelius J, Xu ZZ, Morton J, Garg N, Jansson JK, Knight, R. Microbiome wide associations studies link dynamic microbial consortia to disease. *Nature*, 535: 94-103, 2016.

doi: 10.1038/nature18850

[48] Degnan PH, Pusey AE, Lonsdorf EV, Goodall J, Wroblewski EE, Wilson ML, Ochman H. Factors associated with the diversification of the gut microbial communities within chimpazees from Gombe National Park. *Proceedings of the National Academy of Sciences*, 109: 13034-13039, 2012.

doi: 10.1073/pnas.1110994109

[49] Bonder MJ, Kurilshikov A, Tigchelaar EF, Mujagic Z, Imhann F, Vila AV, Deelen P, Vatanen T, Schirmer M, Smeekens SP, Zhernakova DV, Jankipersadsing SA, Jaeger M, Oosting M, Cenit MC, Masclee AA, Swertz MA, Li Y, Kumar V, Joosten L, Harmsen H, Weersma RK, Franke L, Hofker MH, Xavier RJ, Jonkers D, Netea MG, Wijmenga C, Fu J, Zhernakova A. The effect of host genetics on the gut microbiome. *Nature Genetics*, 48: 1407-1412, 2016.

doi: 10.1038/ng.3663

[50] Barko PC, McMichael MA, Swanson KS, Williams DA. The gastrointestinal microbiome: A review. *Journal of veterinary internal medicine*, 32: 9–25, 2018.

doi: 10.1111/jvim.14875

[51] Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science*, 326: 1694-1697, 2009.

doi: 10.1126/science.1177486

[52] Zhang Z, Li D, Refaey MM, Xu W. High spatial and temporal variations of microbial community along the southern catfish gastrointestinal tract: Insights into dynamic food digestion. *Frontiers in microbiology*, 8: Article 1531, 2017.

doi: 10.3389/fmicb.2017.01531

[53] Bobbie C, Mykytczuk N, Schulte A. Temporal variation of the microbiome is dependent on body region in a wild mammal (*Tamiasciurus hudsonicus*). FEMS Microbiology Ecology, 93.fix081, 2017.

doi: 10.1093/femsec/fix081

[54] Ji BW, Sheth RU, Dixit PD. Wang HH, Vitkup D. Quantifying spatiotemporal variability and noise in absolute microbiota abundances using replicate sampling. *Nature Methods*, 16: 731-736, 2019.

doi: 10.1038/s41592-019-0467-y

[55] Falony G, Vieira-Silva S, Raes J. Richness and ecosystem development across faecal snapshots of the gut microbiota. *Nature Microbiology*, 3: 526-528, 2018.

doi: 10.1038/s41564-018-0143-5

[56] Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, Gaskins HR, Stumpf RM, Yildirim S, Torralba M, Gillis M, Wilson BA, Nelson KE, White BA, Leigh SR. Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME journal*, 7: 1344-1353, 2013.

doi: 10.1038/ismej.2013.16

[57] Nakamura N, Amato KR, Garber P, Estrada A. Mackie RI, Gaskins HR. Analysis of the hydrogenotrophic microbiota of wild and captive black howler monkeys (*Alouatta pigra*) in palenque national park, Mexico. *American Journal of Primatology*, 73: 909-919, 2011.

doi: 10.1002/ajp.20961

[58] Dhanasiri AKS, Brunvold L, Brinchmann MF. Kornes K, Bergh O, Kiron V. Changes in the Intestinal Microbiota of Wild Atlantic cod Gadus morhua L. Upon captive rearing. microbial ecology, 61: 20-30, 2011.

doi: 10.1007/s00248-010-9673-y

[59] Reese AT, Dunn RR. Drivers of microbiome biodiversity: A review of general rules, feces, and ignorance. *MBio*, 9: e01294-18, 2018.

doi: 10.1128/mBio.01294-18

[60] Restrepo CA, Botero-Botero Á. Trophic ecology of neotropical otter *Lontra longicaudis* (Carívora, *Mustelidae*) in La Vieja river, alto Cauca, Colombia. *Boletín Científico. Centro de Museos. Museo de Historia Natural*, 16: 207-214, 2012.

http://www.scielo.org.co/scielo.php?pid=S0123-30682012000100017&script=sci_abstract&tlng=en

[61] Ingala MR, Simmons NB, Wultsch C, Krampis K, Speer KA, Perkins SL. Comparing Microbiome Sampling Methods in a Wild Mammal: Fecal and Intestinal Samples Record Different Signals of Host Ecology, Evolution. *Frontiers in Microbiology*, 9:803, 2018.

doi: 10.3389/fmicb.2018.00803

[62] Lindahl, T. Instability and decay of the primary structure of DNA. *Nature*, 362: 709-715, 1993.

doi: 10.1038/362709a0

Comparación de bacterias intestinales entre nutrias neotropicales silvestres y en cautiverio

Resumen: La nutria neotropical (Lontra longicaudis) es considerada una especie emblemática para la conservación de los ecosistemas en los que reside y actualmente se encuentra en estado vulnerable. Como una estrategia de conservación para esta especie se han propuesto programas de rehabilitación, crianza y reintroducción de individuos cautivos. Sin embargo, es probable que el ambiente y la dieta en cautiverio resulten en comunidades microbianas intestinales que difieren de las de animales silvestres. Las comunidades microbianas tienen un papel importante en el desempeño fisiológico de un animal. Para determinar las diferencias entre las comunidades microbianas intestinales de nutrias silvestres y en cautiverio, se determinó la estructura y diversidad de estas comunidades microbianas intestinales usando rDNA 16S como marcadores moleculares. El DNA total fue aislado de muestras fecales de animales salvajes de la cuenca del río La Vieja y de animales en cautiverio del zoológico de Cali. Tal como se esperaba, las comunidades bacterianas intestinales de los animales cautivos convergieron en una estructura similar y su diversidad bacteriana fue significativamente menor que la encontrada en animales salvajes.

Palabras clave: comunidades bacterianas intestinales; *Lontra longicandis*; perfil molecular PCR-DGGE; nutrias silvestres y en cautiverio.

Comparação de bactérias intestinais de lontras neotropicais silvestres e em cativeiro

Resumo: As lontras neotropicais (Lontra longicaudis) é considerada uma espécie emblemática para a conservação dos ecossistemas nos quais reside e atualmente encontra-se em estado vulnerável. Como uma estratégia de conservação para esta espécie se proponen programas de reabilitação, criação e reintrodução de indivíduos cautivos. Entretanto, é provável que o ambiente e a dieta em cativeiro resultem em comunidades microbianas intestinais que se diferenciam das comunidades de animais silvestres. As comunidades microbianas têm um papel importante no desempenho fisiológico de um animal. Para determinar as diferenças entre as comunidades microbianas intestinais de lontras silvestres e em cativeiro, se determinou a estrutura e diversidade de essas comunidades microbianas intestinais usando rDNA 165 como marcadores moleculares. O DNA total foi isolado de amostras fecais de animais selvagens da bacia do rio La Vieja e de animais em cativeiro do zoológico de Cali. Como esperado, as comunidades bacterianas intestinais dos animais em cativeiro convergiram em uma estrutura similar e sua diversidade bacteriana foi significativamente menor que a encontrada em animais selvagens.

Palavras-chave: Comunidades bacterianas intestinais; *Lontra longicaudis*; perfil molecular PCR-DGGE; lontras silvestres e em cativeiro.

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